



Complete Genome Sequence of *Lactobacillus plantarum* subsp. *plantarum* Strain LB1-2, Isolated from the Hindgut of European Honeybees, *Apis mellifera* L., from the Philippines

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ABSTRACT *Lactobacillus plantarum* subsp. *plantarum* strain LB1-2, isolated from the hindgut of European honeybees in the Philippines, is active against *Paenibacillus larvae* and has broad activity against several Gram-positive and Gram-negative bacteria. The complete genome sequence reported herein contains gene clusters for multiple bacteriocins and extensive gene inventories for carbohydrate metabolism.

Lactobacillus spp. belong to a group of lactic acid bacteria whose members have a low GC content, are Gram positive, and are facultative anaerobic to microaerophilic. This group elicits beneficial effects toward gastrointestinal health and has long been considered a gold standard in probiotic preparations (1). The lactobacilli genomes have shown a high degree of plasticity (2), providing the group competitive advantages in colonizing a wide range of ecological environments, such as in humans, plants, and animals, including honeybees (3). *Lactobacillus plantarum* subsp. *plantarum* LB1-2 was isolated from the hindgut of European honeybees, *Apis mellifera* L., and found to inhibit the growth of *Paenibacillus larvae*, the causative agent of American foul brood disease in honeybees (4). The 16S rRNA sequence was found to be 99.0% identical to that of *L. plantarum* LP11F (4), which was isolated from pig gut (5).

Genomic DNA of LB1-2 was extracted and sequenced at Macrogen, Inc. (Seoul, Republic of Korea). Sequencing libraries from the genomic DNA extracts were prepared using the SMRT Cell 8Pac version 3.0 and the DNA polymerase binding kit P6 and sequenced using PacBio RS II technology (Pacific BioSciences, USA). The PacBio reads (1,255,116,911 bp; 370,050 reads) were *de novo* assembled into contigs using the Hierarchical Genome Assembly Process version 3.0 (HGAP3), and the ends of each contig were overlapped to the final genome, which comprised 3,541,869 bp with a GC content of 44.14% and an average sequencing depth of 260×. The assembled genome yielded four replicons composed of one chromosome (3,359 kbp) and three plasmids (117.3, 56.9, and 8.2 kbp). A total of 3,400 predicted coding DNA sequences, 16 rRNAs, and 77 tRNAs were annotated using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP) (https://www.ncbi.nlm.nih.gov/genome/annotation_prok). Species identity was established by calculating the average nucleotide identity (ANI) and *in silico* digital DNA-DNA hybridization (dDDH) using the ANI Calculator (6) and the Genome-to-Genome Distance Calculator version 2.1 (7), respectively, against previously sequenced genomes in the GenBank database. Secondary metabolites were predicted using antiSMASH version 4 (8), while bacteriocins was predicted using BACTERIOCIN GENOME mining tool version 3 (BAGEL3) (9).

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The genome of LB1-2 has ANI and dDDH values of 99.33% and 96.99% (75.51% formula 2), respectively, with *L. plantarum* subsp. *plantarum* ATCC 14917^T, which supports the systematic placement of LB1-2 within this species. The LB1-2 genome encodes gene clusters for the biosynthesis of fusaricidin, terpene, and exopolysaccharides. The genome revealed the presence of gene clusters homologous to multiple bacteriocins, such as plantaricins A, EF, and JK. The genome also revealed a repertoire of genes encoding sugar transport and utilization. The plasmids pLB1-2A, pLB1-2B, and pLB1-2C encode a type I restriction modification system, a type IV secretion system, and hypothetical proteins, respectively. These findings suggest that these gene inventories could play an important part in the interaction of *L. plantarum* subsp. *plantarum* LB1-2 with its insect host, the nectar substrate, and bee pathogens. These findings highlight the potential use of *L. plantarum* subsp. *plantarum* LB1-2 as a honeybee probiotic.

Accession number(s). This whole-genome shotgun project has been deposited in DDBJ/ENA/GenBank under the accession numbers CP025991 for the chromosome and CP025992, CP025993, and CP025994 for the plasmids pLB1-2A, pLB1-2B, and pLB1-2C, respectively.

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REFERENCES

- Papadimitriou K, Zoumpopoulou G, Foligné B, Alexandraki V, Kazou M, Pot B, Tsakalidou E. 2015. Discovering probiotic microorganisms: *in vitro*, *in vivo*, genetic and omics approaches. *Front Microbiol* 6:58. <https://doi.org/10.3389/fmicb.2015.00058>.
- Molenaar D, Bringel F, Schuren FH, De Vos WM, Siezen RJ, Kleerebezem M. 2005. Exploring *Lactobacillus plantarum* genome diversity by using microarrays. *J Bacteriol* 187:6119–6127. <https://doi.org/10.1128/JB.187.17.6119-6127.2005>.
- Olofsson TC, Alsterfjord M, Nilson B, Butler E, Vasquez A. 2014. *Lactobacillus apinorum* sp. nov., *Lactobacillus mellifer* sp. nov., *Lactobacillus mellis* sp. nov., *Lactobacillus melliventris* sp. nov., *Lactobacillus kimbladii* sp. nov., *Lactobacillus helsingborgensis* sp. nov. and *Lactobacillus kullabergensis* sp. nov., isolated from the honey stomach of the honeybee *Apis mellifera*. *Int J Syst Evol Microbiol* 64:3109–3119. <https://doi.org/10.1099/ijs.0.059600-0>.
- Montecillo AD, Sabino NG, Fajardo A, Jr, Cervancia CR, Aborot ZA, Perdigon KMD, Lantican NB. 2014. Screening of lactic acid bacteria from *Apis mellifera* L. and *Trigona* spp. against *Paenibacillus* larvae (white) causing American foul brood disease of honeybees. *Philipp Ent* 28:32–42.
- De Angelis M, Siragusa S, Caputo L, Ragni A, Burzigotti R, Gobetti M. 2007. Survival and persistence of *Lactobacillus plantarum* 4.1 and *Lactobacillus reuteri* 3S7 in the gastrointestinal tract of pigs. *Vet Microbiol* 123:133–144. <https://doi.org/10.1016/j.vetmic.2007.02.022>.
- Goris J, Konstantinidis KT, Klappenbach JA, Coenye T, Vandamme P, Tiedje JM. 2007. DNA–DNA hybridization values and their relationship to whole-genome sequence similarities. *Int J Syst Evol Microbiol* 57:81–91. <https://doi.org/10.1099/ijs.0.64483-0>.
- Meier-Kolthoff JP, Auch AF, Klenk HP, Göker M. 2013. Genome sequence-based species delimitation with confidence intervals and improved distance functions. *BMC Bioinformatics* 14:60. <https://doi.org/10.1186/1471-2105-14-60>.
- Blin K, Wolf T, Chevrette MG, Lu X, Schwalen CJ, Kautsar SA, Suarez Duran HG, de los Santos ELC, Kim HU, Nave M, Dickschat JS, Mitchell DA, Shelest E, Breitling R, Takano E, Lee SY, Weber T, Medema MH. 2017. antiSMASH 4.0—improvements in chemistry prediction and gene cluster boundary identification. *Nucleic Acids Res* 45:W36–W41. <https://doi.org/10.1093/nar/gkx319>.
- van Heel AukeJ, de Jong Anne, Montalbán-López Manuel, Kok Jan, Kuipers OscarP. 2013. BAGEL3: automated identification of genes encoding bacteriocins and (non-)bactericidal posttranslationally modified peptides. *Nucleic Acids Res* 41:W448–W453. <https://doi.org/10.1093/nar/gkt391>.